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## Selective inhibitory synapse loss in chronic cortical slabs: a morphological basis for epileptic susceptibility

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Electron microscopic examination of pyramidal neurones at the edges of chronic slabs of cerebral cortex in the cat revealed a selective loss of inhibitory (symmetric axosomatic) synapses compared with pyramidal neurones in the centers of the slabs. It appears likely that the neurons at the edges, which retain excitatory input (asymmetric axodendritic synapses) in the neuropil, but totally lack the somatic inhibitory input, act as the focus for the prolonged seizure activity which occurs in chronic cortical slabs.

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Un examen, au microscope électronique, de neurones pyramidaux sur les arêtes de tranches chroniques du cortex cérébral de chat a montré qu'on y relevait une perte sélective de synapses inhibitrices (axosomatiques symétriques) comparé aux neurones pyramidaux des centres des tranches. Il semble vraisemblable que les neurones des arêtes, qui conservent une entrée excitatrice (synapses axodendritiques asymétriques) dans le neuropile, mais qui sont totalement dépourvus d'entrée inhibitrice somatique, agissent à titre de foyer de l'activité de crise prolongée qui se produit dans les tranches corticales chroniques.

[Traduit par le journal]

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## Introduction

Chronically denervated cerebral cortex exhibits prolonged epileptiform activity after electrical or chemical stimulation (Grafstein and Sastry 1957; Reiffenstein 1979). The suggestion that a generalized loss of recurrent collaterals to inhibitory neurones was responsible for the prolonged seizures (Krnjević *et al.* 1970*b*) was recently shown to be incorrect (Reiffenstein 1979). Although inhibition and excitation in the middle of chronic slabs of cortex is normal (Krnjević *et al.* 1970*a*, 1970*b*), there is a decrease in total  $\gamma$ -aminobutyric acid (GABA) content of chronic cortical slabs (Reiffenstein and Neal 1974; Koyama and Jasper 1977), which suggested that there is a localized deficit of inhibitory transmission, possibly at the edges of the slabs. A deficit of this sort has recently been demonstrated at the edges of alumina gel epileptic lesions (Ribak *et al.* 1979). This report is a qualitative description of an electron microscopic examination of synapses at the edges of chronic cortical slabs.

## Methods

Chronic cortical slabs were prepared in suprasylvian gyri of four cats according to the method of Burns (Krnjević *et al.* 1970*a*; Reiffenstein 1979). At 9–10 weeks, when the epileptic susceptibility is fully developed (Sharpless and Halpern 1962), the cats were again anesthetized with pentobarbital and the brains fixed by intracardiac perfusion of aldehydes (Ribak *et al.* 1979). Blocks of tissue were cut from both contralateral suprasylvian gyrus and chronic slabs. These were osmicated, *en bloc* stained with uranyl acetate, dehydrated, and embedded in epon resin. Semithin, 1- $\mu$ m sections were taken from each block to identify cell layers and the edges of each slab (or the homotopic area in the contralateral cortex). Thin sections for electron microscopy were obtained from layers V and VI, to compare slab edges, the middle of the slabs, and normal cortex.

## Results

Pyramidal neurones with their long apical dendrites could easily be identified in the contralateral cortex and throughout the chronic slabs. In contralateral cortex axosomatic symmetric synapses (inhibitory) were regularly present on pyramidal cells, and in the adjacent neuropil there were numerous terminals making synapses (mostly asymmetric, which is the type correlated with excitatory function) with dendrites and dendritic spines (Fig. 1). The number of these asymmetric synapses is undoubtedly diminished, since input fibres from the opposite cortex (which form purely excitatory, asymmetric synapses; for a review see Colonnier 1981) have been severed by the undercut. In spite of the lesion, however, there is no obvious lack of asymmetric synapses, and astroglia are relatively infrequent. In contrast, astroglia were numerous throughout the chronically isolated slabs (Figs. 2–8), particularly near the edges (i.e., the isolation cut). Within 100  $\mu$ m of the

edges the neurones were devoid of all axosomatic synapses (Figs. 3–6) and the somata of the neurones were almost completely surrounded by layers of astroglia. The terminals which were present in this part of the slab appeared to be making asymmetric synapses with dendrites or dendritic spines (Figs. 2, 4, and 6). Neurones in the middle of the slabs had approximately normal numbers of inhibitory axosomatic synapses, while neurones 1 mm from the edges had intermediate numbers of axosomatic synapses, and also more terminals which were apposed to these somata compared with neurones near the edges (Figs. 7 and 8). Fewer astrocytes were present in the center than at the edges, and in contrast the neuronal somata in the center were not completely surrounded by them. Axodendritic synapses in the neuropil were present all across the isolated slabs, even within 100  $\mu$ m from the edges of the slabs (Figs. 2–8).

## Discussion

In the chronic slabs of cerebral cortex there is a total loss of synapses located on pyramidal cell bodies close to the edges of the slabs. Synapses normally present at this site are always of the symmetrical type (Colonnier 1968, 1981). Terminals forming these synapses in monkeys and rats contain glutamic acid decarboxylase (GAD), and these synapses are therefore correlated with the mediation of GABAergic inhibition in the cerebral cortex (Ribak *et al.* 1979). Ribak *et al.* (1982) have recently extended these studies to show that virtually all symmetric cortical synapses are GABAergic. In contrast to their total loss of somatic inhibitory input, these neurones at the edges of the slabs appear to have maintained an important excitatory input from axodendritic synapses in the neuropil. Moreover, the somata of these neurones near the edges also were completely wrapped in multiple layers of reactive astrocytes. This appears very similar to the morphology of alumina lesions reported by Ribak *et al.* (1979). Previous investigations of denervated or partly denervated cortex by Colonnier (1964), Szentágothai (1965), Rutledge *et al.* (1972), Rutledge (1978), and Gruner *et al.* (1974) have failed to show these changes; however, the latter three only examined the neuropil and not the somatic synapses. The results of Gruner *et al.* (1974) agree with ours insofar as they observed that asymmetric synapses were present throughout the neuropil, although in reduced numbers compared with normal cortex. This loss of axosomatic synapses has also been reported in cobalt lesions (Fischer 1969) and in human tissue (Brown 1973; Williams *et al.* 1977). At present we have no explanation for the selective loss of inhibitory terminals. Nor do we know whether the inhibitory terminals have merely withdrawn away from the pyramidal cells because of the intensive gliosis at the edges of the slabs,

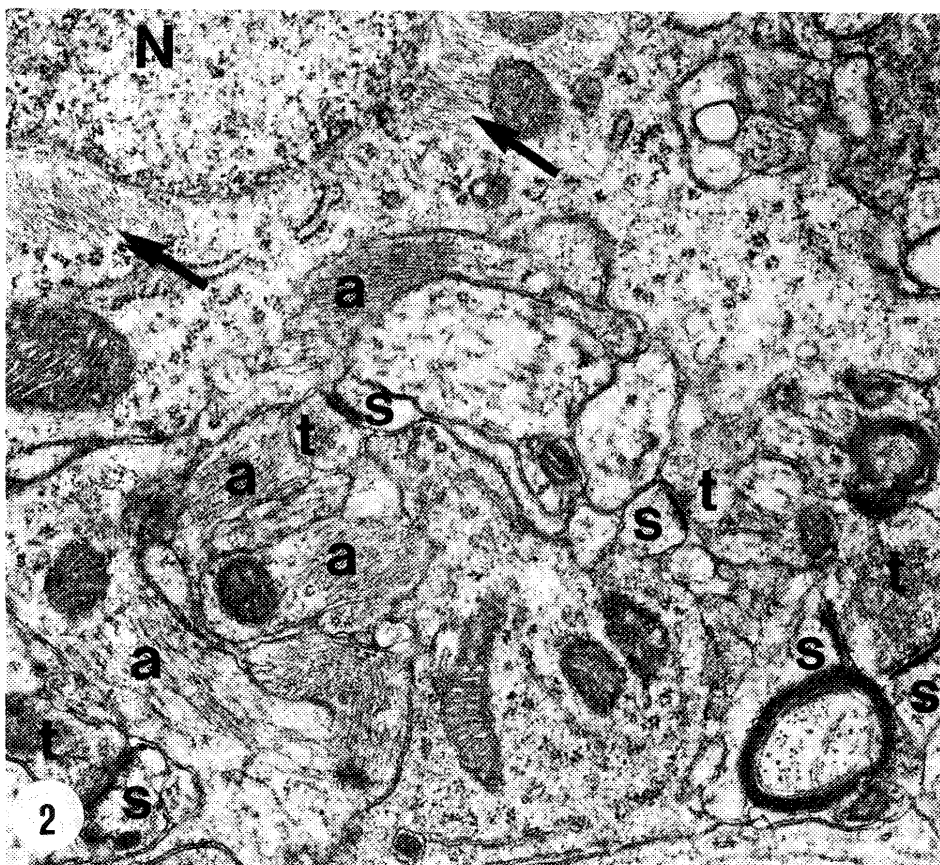
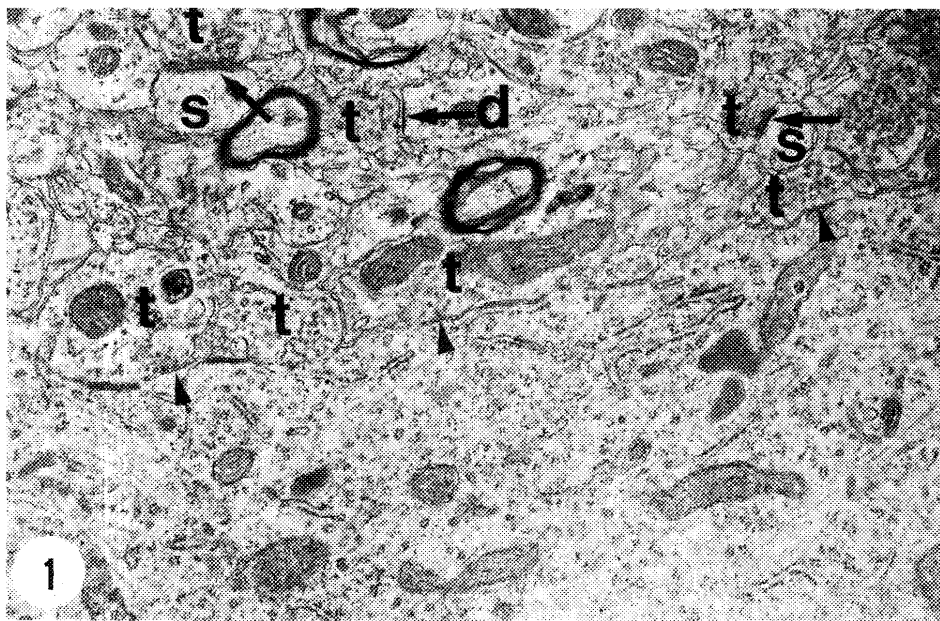


FIG. 1. Electron micrograph (EM) of a portion of a pyramidal cell soma and the adjacent neuropil from intact suprasylvian gyrus. Many terminals (*t*) lie adjacent to this soma, and some form symmetric synapses (arrowheads) with it. Other terminals (*t*) in the neuropil form asymmetric synapses (arrows) with spines (*s*) and dendrites (*d*).  $\times 26\ 000$ . FIG. 2. EM from an isolated slab, located within  $200\ \mu\text{m}$  of the edge of the slab, showing a portion of a reactive astrocyte with its nucleus (*N*) and filaments (arrows). Other astrocytic profiles (*a*) fill the neuropil spaces between axon terminals (*t*) that form asymmetric synapses with dendritic spines (*s*)  $\times 26\ 000$ .

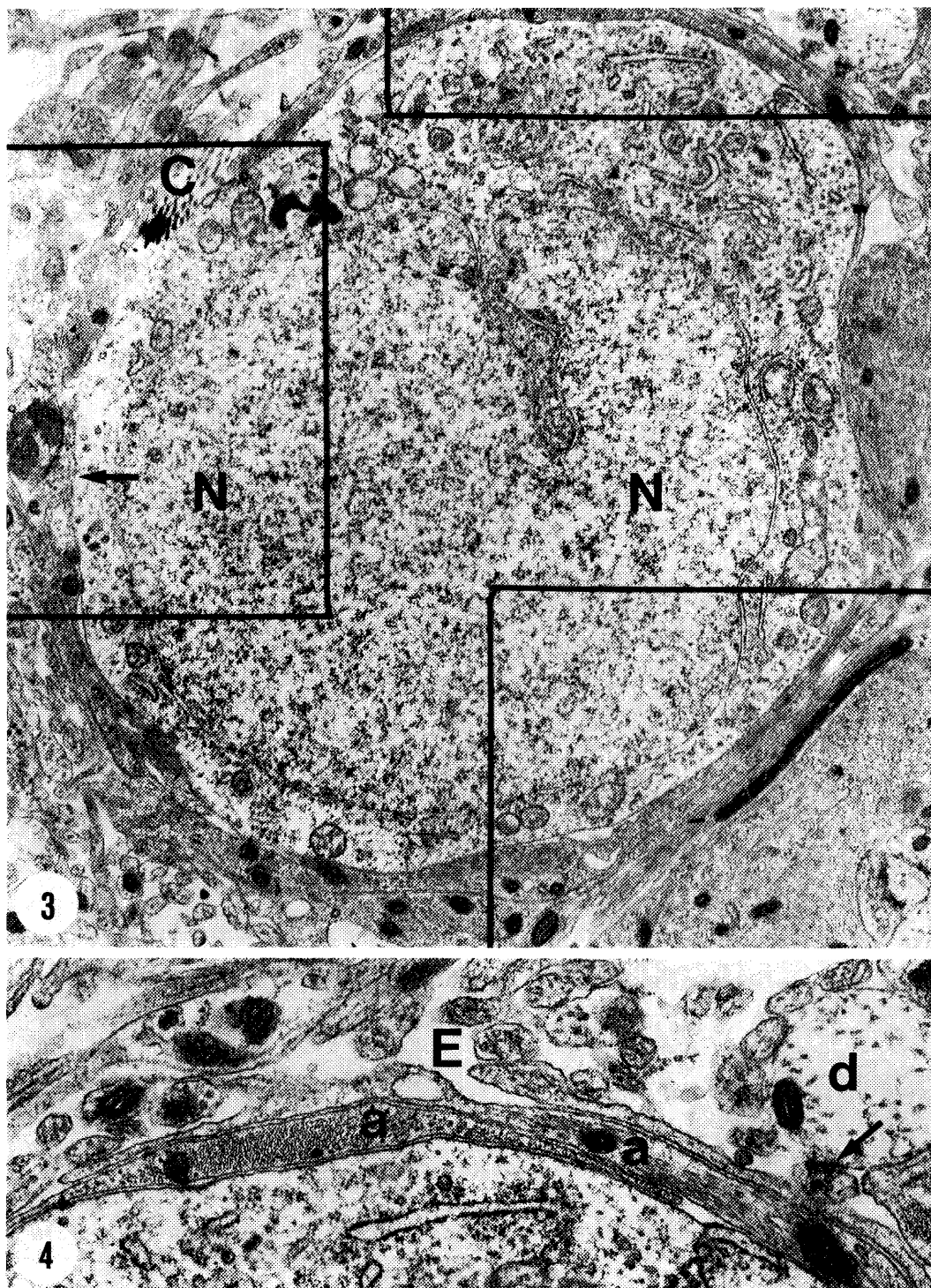


FIG. 3. Low magnification EM of soma of a neurone less than 50  $\mu\text{m}$  from the edge of the slab (to the left). An accumulation of organelles, such as cisternae of Golgi complex, occur at the apical end where the nucleus (N) shows an indentation. This soma is almost entirely surrounded by profiles of reactive astrocytes. This glial covering is only interrupted by collagen fibres (C) and an axon terminal (arrow) that synapses with an adjacent dendrite (see Fig. 6). The boxed areas are shown at higher magnifications in Figs. 3–6.  $\times 11\,000$ . FIG. 4. Higher magnification of area in upper right of Fig. 2. Filaments sectioned either transversely or longitudinally appear in profiles of reactive astrocytes (*a*) that are closely contacting the neuronal soma. A slightly disrupted dendrite (*d*) appears to form a synapse (arrow) with a small axon terminal.  $\times 27\,000$ .



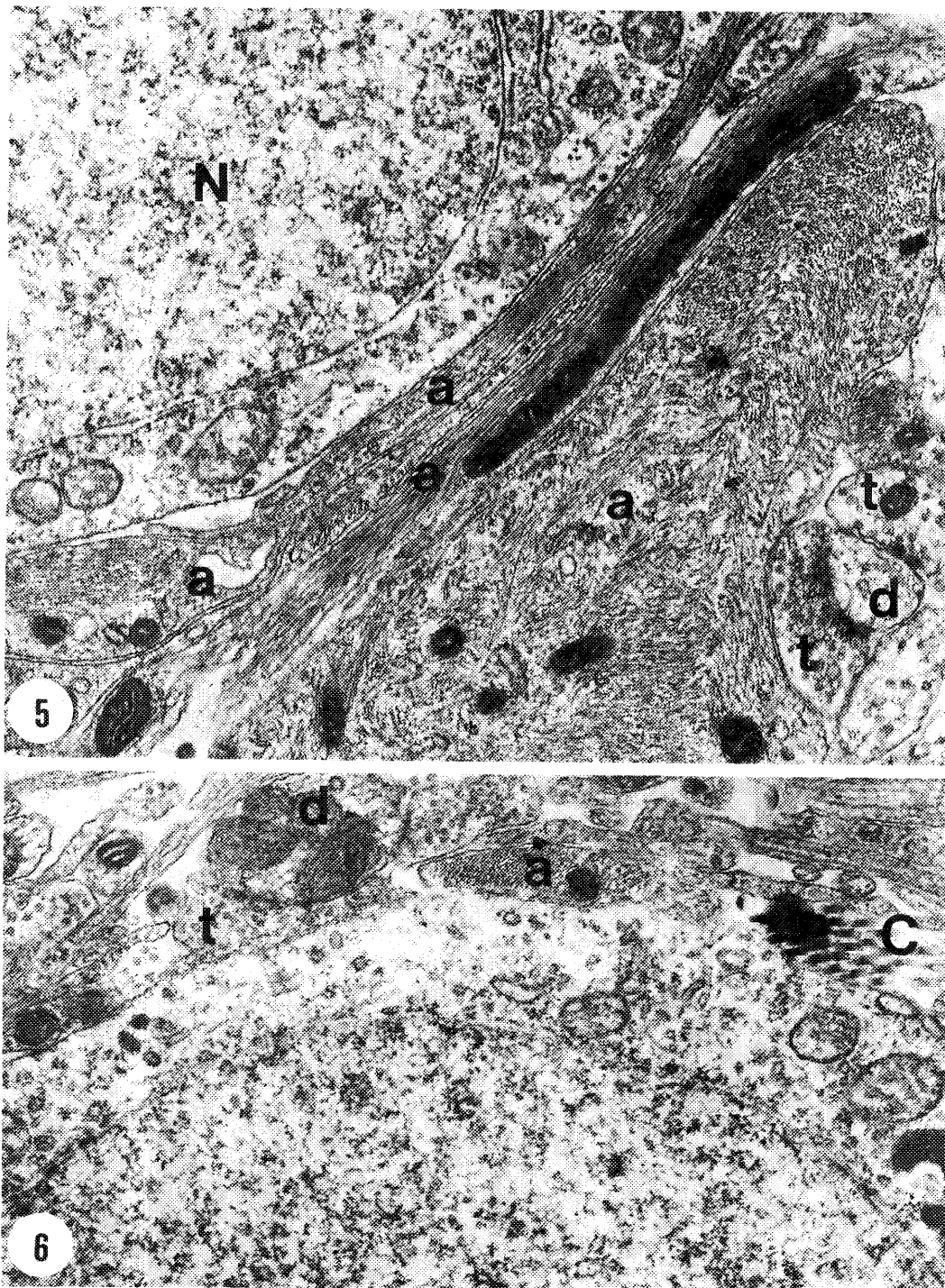


FIG. 5. Higher magnification of area in lower right of Fig. 2. The neuronal nucleus (N) and thin shell of perikaryal cytoplasm are adjacent to several layers of astrocytic processes (a). Two terminals (t) form asymmetric synapses with a dendrite (d).  $\times 26\ 000$ . FIG. 6. Higher magnification of the boxed area of the left of Fig. 2. The sheet of astrocytic processes (a) which surround this soma is interrupted only by collagen fibres (C) and a terminal (t) which formed no observable synaptic specializations with the soma, although it appeared to contact the neighbouring obliquely sectioned dendrite (d).  $\times 26\ 000$ .

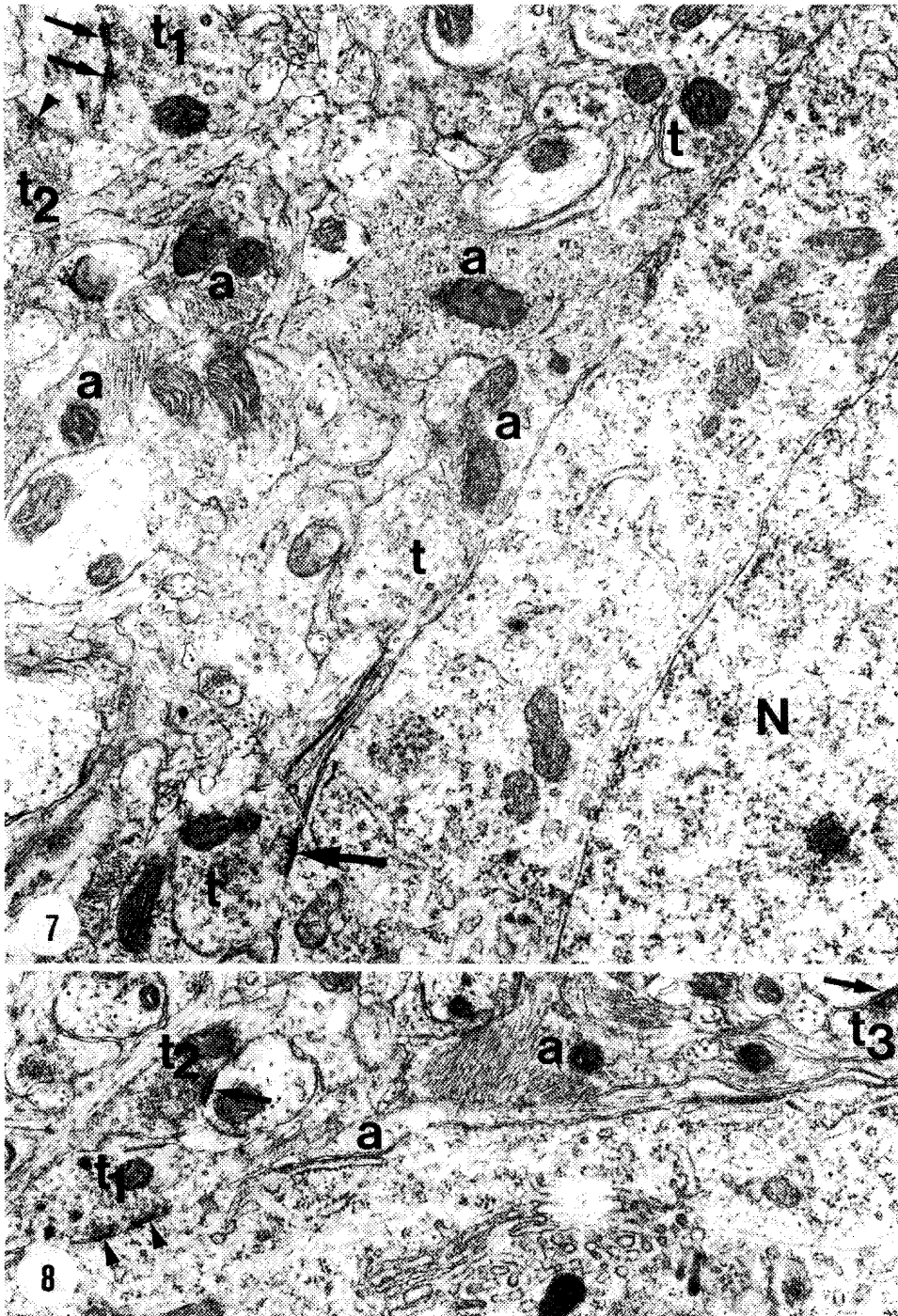


FIG. 7. EM of portions of a pyramidal cell more than 1 mm from the edge of the slab. Part of the nucleus (N) is visible. This soma has three axon terminals (*t*) close to it, and one of these forms a symmetric synapse (arrowhead). Numerous astrocytes (*a*) are present on the neuropil. Two other terminals (*t*<sub>1</sub> and *t*<sub>2</sub>) form asymmetric (arrows) and symmetric (arrowhead) synapses with a dendrite.  $\times 25\,000$ . FIG. 8. Another portion of the same pyramidal cell soma as shown in Fig. 7 shows a terminal (*t*<sub>1</sub>) forming a symmetric axosomatic synapse (arrowheads), with astrocytic processes (*a*) covering the remaining somal surface. In the adjacent neuropil, a bouton *en passant* (*t*<sub>2</sub>) forms an asymmetric synapse (arrow) with a dendrite, and another terminal (*t*<sub>3</sub>) forms a similar type synapse (arrow).  $\times 22\,500$ .

or if there has been a loss of aspiny stellate cell bodies. The latter has been reported in some human lesions (Braak and Gobel 1978). Nevertheless, it seems probable that the pyramidal cells at the edges are acting as the focus for the prolonged epileptiform discharge which is characteristic of chronically denervated slabs of cerebral cortex. Other nearby pyramidal cells, with partial losses of inhibitory synapses, are probably more easily recruited into the activity of the focus than neurones in the middle of the slab, or in normal cortex. On the basis of the decrease in GABA content (Reiffenstein and Neal 1974; Koyama and Jasper 1977) one would predict that the *total* loss of inhibitory synapses throughout the slab is about 30%. The absence of major losses of inhibitory or excitatory inputs to the neurones in the middle of the slabs does at least provide an explanation why most other investigations have found an apparent normalcy of most neurones in epileptic chronic cortical slabs (e.g., Krnjević *et al.* 1970a, 1970b; Reiffenstein 1979). Quantitative data for the distributions of inhibitory and excitatory synapses in these preparations, and also in slabs of 1–2 weeks duration (when the slabs do not yet show prolonged seizures (Sharpless and Halpern 1962)) are currently being accumulated.

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